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TITLE: Genetic Influences on Toxicity and Prognosis in Women Treated with Breast-Conserving Surgery and Radiation Therapy

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13. ABSTRACT (Maximum 200 Words)

Women with earlier stage breast cancer who receive breast conserving surgery and radiation therapy have a generally good prognosis. However, among 15-20% of these women, breast cancer recurs, and a similar proportion of women also experience severe toxicity with radiation therapy. It is possible that inter-individual differences in capabilities of both tumor and normal cells to protect themselves from radiation-induced damage, and to repair that damage if it does occur, will influence recurrence and toxicity. This variability common genetic polymorphisms. This study is conducted in a well-characterized cohort of women who had breast-conserving surgery followed by radiation therapy, and in whom skin reactions were measured and noted. We are extracting DNA from blood to determine genetic polymorphisms in a number of genes that may be important in response to treatment. By conducting follow-up on the women in the study, we will be able to determine how variability in genes that protect cells from damage and in those that repair DNA damage will affect both breast cancer recurrence and toxicity experienced. Follow-up is ongoing, through clinic visits, letters, and home visits, and in the next year, we will correlate genotyping results with toxicity.

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Table of Contents

INTRODUCTION: Women with earlier stage breast cancer who receive breast conserving surgery and radiation therapy have a generally good prognosis. However, among 15-20% of these women, breast cancer recurs, and a similar proportion of women also experience severe toxicity with radiation therapy. It is possible that inter-individual differences in capabilities of both tumor and normal cells to protect themselves from radiation-induced damage, and to repair that damage if it does occur, will influence recurrence and toxicity. Activity of many of the proteins involved in these processes are determined by common inborn genetic differences, termed genetic polymorphisms. We conducted a pilot study to determine if this were the case, and although the study population was mixed in stage at diagnosis and treatments received, we found that women with variant alleles that would allow more treatment-generated reactive intermediates to reach tumor cells had better survival.
We are conducting the present study in a well-characterized cohort of women who had breast-conserving surgery followed by radiation therapy, and in whom skin reactions were measured and noted. We are extracting DNA from blood to determine genetic polymorphisms in a number of genes that may be important in response to treatment. By conducting follow-up on the women in the study, we will be able to determine how variability in genes that protect cells from damage and in those that repair DNA damage will affect both breast cancer recurrence and toxicity experienced.

BODY: Research accomplishments associated with each Task outlined in the Statement of Work will be addressed within the context of each of the objectives.

Technical Objective 1 Follow-up of breast cancer patients of the parent study regarding therapy outcome and survival and data collection.

The approved subcontract to the DKFZ was received in March. Therefore, the field work could only commence as of April 2003 and should be completed by the end of 2004.

Task 1: Months 1-2: Organization of recontact with patients through different sources, development of clinical data forms and questionnaire, and establishment of data base.

Recruitment in the participation clinics

The personal data of the 478 patients were assembled from the four participating clinics in northern Baden-Württemberg for the follow-up. To achieve comparable follow-up interval between the follow-up examination and end of radiotherapy, the patients are divided into two groups for follow-up (table 1.)

	Group I	**Group II**
	Radiation in 1998/99	Radiation in 2000/01
Clinic	Number of patients	Number of patients
University of Heidelberg	145	124
St. Vincentius Hospital Karlsruhe	60	49
Municipal Hospital Karlsruhe	48	24
University of Mannheim	20	8

Total number of patients	273	205
Follow-up to be conducted in year	2003	2004
Interval since radiation	4 -5 years	3.5 -5 years

The first group will be contacted and examined in 2003, where possible. The second group will be included and examined in 2004.

It was first necessary to identify the patients who can be recruited during after-care in the 4 participating clinics. After-care is organized differently in the 4 participating clinics and the identification of patients required extensive search at different units and data bases. The study patients who use the aftercare facilities regularly at the University Clinic Heidelberg and in the two clinics in Karlsruhe were identified.

Table 2. Participation of the patients of group I in the after-care consultation at the clinics in Heidelberg and in Karlsruhe

Clinic	Number of patients in the after-care consultation			
	regular	not regular	never	unknown
University of Heidelberg 3 consultations combined	44	53	48	
St. Vincentius Ka	29	18	13	
Municipal Hospital Ka	8	13	22	5

Less than half of the study patients make use of the after-care consultations at the participating clinics, thus it was agreed upon that all patients will be contacted by letter through the participating clinics in order to invite them specifically to a follow-up examination and participation in the follow-up study. A patient information sheet was attached to this letter.
Patients of the University hospital in Heidelberg who reply to the invitation are given an appointment by the project radio-oncologist, either at the clinic or at home. The project radiologist then conducts the follow-up examination and the study data collection.
Patients from the other hospitals in Karlsruhe who consent to participation are examined at the clinic in the course of the after-care consultations. The project radiologist collects the questionnaire and clinical data.
All physicians at the two clinics in Karlsruhe who attend to after-care consultation were informed about study procedures and made familiar with the documents. They are responsible for completing the data forms on late toxicity. All three clinics have been provided with study material and the organizational procedure of patient recruitment has been integrated into the normal clinical routine.
The letters of invitation were sent out in June to the patients from the University Heidelberg and in July to the patients from Karlsruhe.

Other recruitment procedures
Dr. Helmbold, a radio-oncologist who was responsible for recruitment, will approach by telephone all patients of Heidelberg University Women's Clinic and the two hospitals in

Karlsruhe who do not make an appointment for follow-up examination.

Informed consent of the patients
The patient information sheet as well as formal consent and assignment contract of blood samples are already available and have been approved by the ethics committee of the University of Heidelberg (Appendix 1 and 2)
For the follow-up recruitment at the participating Mannheim University Clinic, a separate approval by the local ethics committee is required and the application is still under examination.

Development of clinical data forms and questionnaire
A standardized documentation of late toxicity was established in agreement with the senior consultants of the radiation departments in the participating clinics. A classification schema was created that corresponds in general to the classification of RTOG/EORTC in the version of 1993, published by Seegenschmiedt, 1999. The classification was modified using criteria of the Lent-Soma Scores and records the examination result in five grades, in scores of 0 to 4. Included in the scoring are (1) the general condition, (2) radiogenic changes in organs like heart and lungs and (2) radiogenic changes of the breast.

A clinical data sheet was developed to obtain data on occurrence of a local or regional recurrence, histology, degree of histological differentiation, state of receptor as well as therapy of local recurrence, metastases distant from the primary tumour or a second carcinoma, and cause of death as well as the last observation date.

A patient questionnaire modified from the questionnaire employed in the parent study will be used to obtain information on relevant demographic and other risk factors.

Task 2: Months 3-24: Recruitment of patients through different sources, perform follow-up examination, obtain informed consent, collect clinical data, complete questionnaire, data input
Patient recruitment at the University Hospital in Heidelberg and in the hospitals in Karsruhe, as described, is ongoing and will be continued in the upcoming year.

Task 3: Months 24-36: Double data entry with ongoing quality control and plausibility checks
In preparation for this task, we have developed a database for entry of the questionnaire data and clinical data on toxicity and outcome. Data collected will be double entered. Data checks and data cleaning for data collected and entered will be performed. Study data will be entered on an ongoing basis.

Task 4: Months 30-36 Perform statistical data analysis; initial descriptive analyses, study of main effects of data derived from questionnaire.
To be completed in the third year.

Technical Objective 2 Evaluation of the effect of genetic polymorphisms in certain candidate genes (i.e. alleles that confer reduced protection from ROS damage and variants in DNA repair genes) and outcomes; i.e., breast cancer recurrence and severe skin toxicity.

Task 1: Months 3-6 DNA extraction and shipment of aliquot
Genomic DNA was extracted in the Heidelberg laboratory (Flexigene DNA isolation kit, Quiagen Systems) and an aliquot shipped to Dr. Ambrosone's laboratory. Genotyping will be performed over the next year. Blood samples are available from 447 patients.

Task 2: Months 26-30 Perform DNA analysis for genetic polymorphisms in genes that confer reduced protection from ROS damage, e.g. *MnSOD, GPX1, CAT, GSTT1, GSTM1, GSTA1, GSTP1*, and in DNA repair genes, associated with risk of cancer and/or ionizing radiation sensitivity e.g. *XRCC1, XRCC3, Ligase IV, XPD, APE1*
Genotyping will begin in the upcoming year of the study.

Task 3: Months 31-36 Merge data from laboratory results with questionnaire database. Perform statistical analysis for main effects of polymorphisms on outcomes.
To be completed in the third year.

REPORTABLE OUTCOMES: None to date, still in data collection phases.

CONCLUSIONS: To date, we have been proceeding to recontact patients and conduct follow-up. DNA has been extracted from blood specimens, and samples will be genotyped in the upcoming year.

REFERENCES: None

APPENDICES: None